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Reaction of sugar beet S1 lines and cultivars to different isolates of *Macrophomina phaseolina* and *Rhizoctonia solani* AG-2-2IIIB

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Abstract Interactions of 17 sugar beet lines and cultivars with four isolates of *Macrophomina phaseolina* (the causal agent of charcoal rot) and one isolate of *Rhizoctonia solani* (the causal agent of crown and root rot) were studied in separate experiments under greenhouse conditions. The isolates of *Macrophomina* were taken from their host plants, sugar beet (two isolates), soybean and sesame. In the first experiment, the colonized toothpick was used as inoculum. In the second experiment, six-month-old sugar beet plants were inoculated with barley seeds colonized with *M. phaseolina*. For the inoculation of sugar beet lines with *R. solani,* the colonized corn seeds were used. Root symptoms were recorded four weeks after inoculation, by estimating the proportion of the root surface infected by the pathogens, using a 1–9 standard scale. Our results showed a significant difference among lines and cultivars in their resistance to these two pathogens. Line B8618 was found to be considerably resistant to the isolates of the both pathogens. The inoculation methods of *Macrophomina* isolates had no significant effect on the results. The interaction between isolate and cultivar was not also significant in *Macrophomina*-resistant lines. Therefore, it appears that the response of sugar beet lines to the tested fungal isolates was not differential. These resistant lines showed a high resistance to all the tested *M. phaseolina* isolates. Our results revealed that the *Macrophomina*-resistant lines also showed resistance to *R. solani*. Furthermore, the sugar beet drought tolerant lines (M293, M362 and M345) were susceptible to the tested *M. phaseolina* and *R. solani* isolates.

Keywords Charcoal rot · Drought · *Rhizoctoniasolani* · Sugar beet

Introduction

The causal agents of sugar beet root rot are fungal and bacterial pathogens among which the fungi are more important (Whitney and Duffus 1986). The etiology of sugar beet root rot shows that this disease is caused by different fungi in different regions. Over 30 species of fungi have been reported as the causal agents of root rot around the world (Asher and Hanson 2006) out of which 20 species have been reportedly observed in Iran (Ershad 2009). Among them, *R. solani*, *Pythium aphanidermatum*, *Phytophthora cryptogea*, *Ph. drechsleri*, *Ph. megasperma*, *Ph. nicotiana* and *Fusarium spp*. are widely distributed (Raoufi et al. 2003; Banihashemi 1998; Mahmoudi et al. 2004). On the other hand, the fungus *M. phaseolina* has been reported as a causal agent of root rot in sugar beet fields and roots stored in silo (Asher and Hanson 2006;

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Ershad 2009; Sheikholeslami et al. 1998). *M. phaseolina* and *R. solani* have a wide host range and have been isolated from different crops such as soybean, corn, cotton, sesame, sugar beet, groundnut, potato, melon, watermelon, strawberry, conifers and olive in Iran (Ershad 2009; Mahmoudi et al. 2004). Symptoms of root rot caused by *Macrophomina* and *Rhizoctonia* are dark brown lesions followed by rotting of the root tissue. Similar symptoms of these two pathogens may be due to decomposition and degradation of plant tissues by pectolytic and cellulolytic enzymes produced by the pathogens (Ahmad et al. 2006). Pectin lyase is the main enzyme produced by *R. solani* AG-2-2 isolates in the infected tissues of sugar beet crown and in culture medium (Naito and Sugitomo 1981).

Jones et al. (1998), Vandermark et al. (2000) and Mayek-Perez et al. (2001) showed a great diversity among *Macrophomina* isolates when collected from different hosts and geographical regions. Almeida et al. (2003) studied the genetic diversity of *Macrophomina* isolates for the first time in Brazil and observed differences in the isolates collected from a single plant in addition to the already-proved differences in the isolates of different hosts. Mahmoudi et al. (2005) found great genetic diversity among the Iranian isolates of *R. solani* associated with sugar beet root rot.

Pathogenic variability of isolates of *Rhizoctonia* (Mahmoudi et al. 2004) and *Macrophomina* (Alaghebandzadeh et al. 2008) isolates has been reported previously. This variability may affect the screening of genetic resources of the host. Therefore, it has been recommended to apply highly aggressive isolates in evaluating the resistance of cultivars to *Rhizoctonia* (Windels et al. 1995) and *Macrophomina* pathogens.

(Gaskill et al. 1970) and Hecker and Ruppel (1977) used an aggressive isolate of *R. solani* for finding resistant lines to *Rhizoctonia* root rot. The lines which were resistant to the aggressive isolate of USA displayed resistance to the isolates of Japan as well. Therefore, they concluded that resistance of sugar beet to *R. solani* is not race-specific and that it should be quantitatively inherited. Based on their studies, resistance of sugar beet to *R. solani* shows partial dominance and two major and some modifying genes are involve in its genetic control.

One of the main reasons for limited success in management of such diseases as *Macrophomina* root rot is our limited understanding of the genetic structure of plant pathogen populations and their interaction with hosts (Martin and English 1997;van den Boogert P H J et al. 1998; McDonald and Linde 2002). The stability of host resistance is another important issue which provokes the interest of plant breeders. It is predicable by adequate understanding of the genetic structure of plant pathogen population (McDonald and Linde 2002).

The main objective of the current study was to screen the breeding lines of sugar beet for their resistance to the both pathogens. The reaction of sugar beet S1 pollinator lines and cultivars to different isolates of *Macrophomina* was investigated for the first time in order to identify the genotypes resistant to all pathogenic isolates. These genotypes were then evaluated for their response to a highly-aggressive isolate of *R. solani* AG2-2 (Mahmoudi et al. 2004). Charcoal rot is known as a drought stress-related disease (Beas-Fernandez et al. 2006), so three sugar beet drought tolerant lines were evaluated against *M. phaseolina* isolates in the current study.

Materials and methods

Plant materials

Sugar beet germplasm used in the current study consisted of commercial cultivars of Dorothea, Flores, Jolgeh, Laetitia, Rasta, Shirin, and the S1 lines of B8618, B8633, B8662, B8702, B8706, B8712, B8716, B8723, B8728, B8735, B8738, B8739, B8751, M292, M293, M295, M345 and M362. Also, SB-19 population was used as a *Rhizoctonia*-resistant accession.

Fungal isolates

The isolates of *Macrophomina* used in this study included two isolates from sugar beet (19 and P2M6), one isolate from sesame (KB2) and one isolate from soybean (SK1). One isolate of *R. solani* AG2-2IIIB named Rh133 was used. All isolates were received from the collection of Plant Protection Section, Sugar Beet Seed Institute, Karaj, Iran. The isolates of *Macrophomina* and *Rhizoctonia* were kept on wood and barley seed in long-term storage, respectively.

Evaluation of resistance in greenhouse conditions

Three-forth of the pots was filled with a mixture of soil and peat. Then, sugar beet seeds were sown and covered by sandy soil. After about 1 month, the seedlings were transplanted into larger pots (20 cm diameter) and grown in a greenhouse at 25–27 °C. After 4–5 months, the plants were inoculated with different isolates of *Macrophomina* and *Rhizoctonia*.

In the first experiment, one toothpick colonized with the isolates of *M. phaseolina* was used as inoculum (Schuster et al. 1958; Ahmad et al. 2006). The crown of plants was inoculated with the infested toothpick. Non-colonized toothpicks were used for inoculating control plants. The experiment was carried out in a completely randomized design with 10 replications for each isolate and each replication contained one plant. To record root symptoms, plants were taken out of the soil 4 weeks after inoculation and the proportion of the root surface infected by the pathogen was estimated using a 1–9 standard scale (Buttner et al. 2004). The diseased plants showing the symptoms of root rots were transferred to laboratory and the fungus was isolated from them again.

In the second experiment, the seeds of barley colonized with the isolates of *M. phaseolina* were used as inoculum (Alaghebandzadeh et al. 2008). The inoculum was put around the plants at the depth of 2 cm and covered with soil. Non-inoculated seeds of barley were used for inoculating control plants. The rest of stages were similar to the first experiment.

Corn seeds colonized with Rh133 isolate of *R. solani* were used to evaluate the resistance of the lines to *R. solani* (Mahmoudi et al. 2004). The procedure was similar to the procedure of evaluation for *Macrophomina* in the second experiment. Sugar beet cultivars and lines were compared with each other based upon mean of disease severity (Scholten O E, Panella L W, DeBock T S M, Lange W 2001; Buttner et al. 2004).

Statistical analyses

The data were analyzed by SAS version 11.5. For the traits not normally distributed, the data were transformed using Arc sin. Means comparison for the traits was done by using least significant difference (LSD) ($p < 0.05$).

Results

Evaluation of resistance to *Macrophomina phaseolina*

The interactions among pathogenic isolates of *M. phaseolina* and sugar beet lines and cultivars using toothpick as inoculum are summarized in Table 1. The pathogenic isolates were classified in three levels in terms of their aggressiveness. The Isolate P2M6 was the most aggressive isolate, whereas KB2 hosted by sesame was the least aggressive one. Sugar beet lines showed different responses to the pathogen so that the lines B8618, B8662 and B8751 were found to be highly resistant, while the response of the line M345 was similar to that of the susceptible control cultivar, Jolgeh. The responses of resistant lines to the pathogenic isolates were similar but not differential.

In the second experiment in which barley seeds were used as inoculum, the responses of the lines and cultivars to the pathogenic isolates were similar to those in the first experiment (Table 2). In this experiment, reaction of the lines B8618, B8662, B8751, B8723, B8735, and B8738 was similar to that of Flores, the resistant check for *R. solani*. The interaction of sugar beet lines and cultivars with different isolates of *M. phaseolina* was not significant (Table 2). It means that the reaction of the lines to different isolates of the pathogen was similar and no differential reaction to the isolates was observed among the lines.

Evaluation of resistance to R. solani

Figure 1 shows the results of responses of lines and cultivars to isolate Rh133 according which the cultivars and lines showed significantly different responses at 5 % probability level. Comparison of the mean of disease severity showed that the cultivars Jolgeh and Shirin were the most susceptible and the cultivars Laetitia and Rasta as well as the population SB-19 were the most resistant treatments. The cultivars Jolgeh and Shirin were used as the susceptible control and the SB-19 accession as the resistance source to *R. solani*.

Evaluation of the resistance of sugar beet cultivars and lines to *Macrophomina* and *Rhizoctonia* and their comparison showed that the cultivars Flores, Dorothea, Laetitia and Rasta were resistant to the

Table 1 Disease severity of four isolates of *Macrophomina phaseolina* on 17 sugar beet genotypes inoculated with toothpick (experiment 1)

Isolates	Jolgeh	Flores	M345	M293	M362	B8706	B8728	B8739	B8633
SK1	5.48	3.55	5.63	5.18	5.17	5.62	4,55	4.72	4.18
P2M6	5.63	3.55	5.69	5.56	5.25	5.71	4.97	5.02	4.26
19	5.22	3.55	5.29	4.08	3.96	5.01	3.76	4.04	3.75
KB2	5.30	3.55	5.12	4.05	4.20	5.06	3.96	3.80	3.91
Mean	5.41	3.55	5.43	4.72	4.65	5.35	4.31	4.39	4.02

LSD5 % (Genotypes) = 0.204

LSD5 % (Isolates) = 0.083

LSD5 % (Genotypes × Isolates) = 0.4190

Isolates	B8712	B8702	B8738	B8735	B8723	B8751	B8662	B8618	Mean
SK1	5.12	3.98	4.10	3.62	3.62	3.55	3.55	3.55	4.42
P2M6	5.20	4.66	4.62	4.19	4.18	3.76	3.77	3.55	4.67
19	3.91	3.55	3.78	3.55	3.55	3.55	3.55	3.55	3.97
KB2	3.95	3.55	3.77	3.55	3.62	3.55	3.55	3.55	4.00
Mean	4.***54	3.94	4.07	3.73	3.74	3.60	3.55	3.55	

LSD5 % (Genotypes) = 0.204

LSD5 % (Isolates) = 0.083

LSD5 % (Genotypes × Isolates) = 0.4190

Figures with different letter(s) show significant difference according to Duncan Test ($p < 0.05$)

Table 2 Disease severity of four isolates of *Macrophomina phaseolina* on 17 sugar beet genotypes inoculated with barley seed (experiment 2)

Isolates	Jolgeh	Flores	M345	M293	M362	B8706	B8728	B8739	B8633
SK1	4.62	3.05	4.68	4.48	4.05	3.85	3.70	3.73	3.60
P2M6	4.92	3.09	4.85	4.56	4.12	4.44	3.91	3.57	3.93
19	4.45	3.06	4.48	4.56	3.64	4.24	3.61	3.54	3.40
KB2	4.31	2.99	4.12	4.09	3.27	4.37	3.16	3.52	3.34
Mean	4.57	3.04	4.53	4.42	3.77	4.22	3.59	3.59	3.56

LSD5 %(Genotypes) = 0.274

LSD5 %(Isolates) = 0.117

Isolates	B8712	B8702	B8738	B8735	B8723	B8751	B8662	B8618	Mean
SK1	3.50	3.47	3.41	3.47	3.16	3.40	2.99	2.99	3.65
P2M6	3.79	3.70	3.40	3.65	3.72	3.35	2.99	2.99	3.82
19	3.49	3.42	3.22	3.06	3.27	3.22	3.11	2.99	3.57
KB2	3.44	2.99	3.09	2.99	2.99	3.11	2.99	2.99	3.39
Mean	3.55	3.39	3.28	3.29	3.28	3.27	3.02	2.99	

LSD5 %(Genotypes) = 0.274

LSD5 %(Isolates) = 0.117

Figures with different letter(s) show significant difference according to Duncan Test ($p < 0.05$)

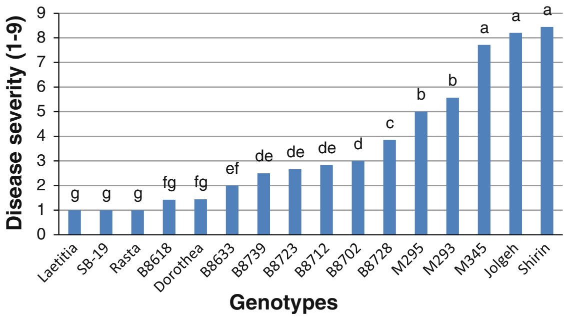

Fig. 1 Comparison of resistance of sugar beet cultivars and lines at maturity stage to *R. solani* (Rh133) in greenhouse conditions at 5 % probability level ($n = 12$)

both pathogens (data not shown). Among the studied lines, the line B8618 was found to be considerably resistant to the both pathogens.

Discussion

The sugar beet charcoal rot disease caused by *M. phaseolina* infects most of the spring and autumn beet cultivation regions, especially in arid environmental conditions in Iran. It is estimated that 30 % of sugar beet cultivation area in Iran is affected by *Rhizoctonia* root rot of sugar beet (Mahmoudi and Soltani, 2005). However, there has been no organized study on the evaluation of the resistance of *Beta* spp. to these two pathogens. Therefore, the current study was undertaken for the first time to test the resistance of 17 cultivars and lines of sugar beet to four isolates of *M. phaseolina* with various aggressiveness levels and one isolate of *R. solani* with high aggressiveness (Mahmoudi et al., 2004). There is great diversity in aggressiveness of different isolates of *Macrophomina* and *Rhizoctonia* (Mahmoudi et al. 2004; Alaghebandzadeh et al. 2008). Therefore, it is recommended to use highly aggressive isolates for screening of resistance resources.

In the greenhouse evaluation of the resistance of sugar beet cultivars and lines to *Macrophomina* root rot, two methods were used: one with toothpick and the other with barley seed as inoculum. In the latter method, it was necessary to remove the soil around the plant and wound the roots to have the inoculum touched to the host which seems to be inaccurate in large scale studies. On the other hand, the extent of the wounding of the roots varied which could affect the results. In addition, it was likely that the seeds roll away the root rhizosphere by irrigation which might make difference in the timing of the infection of individual plants. In the toothpick method, however, the plants received same amount of inoculum by their roots and the depth of scraping was alike. Therefore, given the advantages of toothpick method over barley seed method and its simplicity in inoculation of individual plants, we recommended it to evaluate the resistance of sugar beet genotypes in greenhouse condition.

Since using tolerant and resistant cultivars is the main strategy for controlling this disease, the resistance of 17 lines and commercial cultivars of sugar beet to the disease was studied to identify the likely sources of the resistance. Among these, the lines B8618, B8662 and B8751 were found to be resistant to *M. phaseolina* (Tables 1 and 2). The interaction of isolate and genotype was significant in the first experiment but not in the second experiment. Such an interaction between *Rhizoctonia* isolates and sugar beet has already been reported (Ruppel, 1972; Windels et al. 1995 and Mahmoudi et al. 2004). In previous studies, when the susceptible genotypes were discarded from the statistical analysis, the interaction between isolates and genotypes became insignificant (Windels et al. 1995 and Mahmoudi et al. 2004). In the current study, the reaction of resistant lines was not affected by different isolates of *M. phaseolina*.

Given the high importance of sugar beet cultivation in Iran, it is necessary to consider the limiting factors of its cultivation and to take required actions to overcome them. The main sugar beet growing areas are located in the eastern part of Iran where sugar beet production has some limitations such as drought stress, root rot and rhizomania. Our results showed that drought tolerant pollinators (such as M345 and M293) developed for that region (Ahmadi et al. 2011) were susceptible to charcoal rot. Sugar beet production owes its success to the capability of science in controlling destructive diseases of the plants (Cook and Scott 1993). Sugar beet diseases such as curly top, *Cercospora* leaf spot and root rots were the major constrain for many sugar factories in the U.S. and Europe in early 20th century. Now, it is readily possible for farmers to manage these destructive diseases by the application of resistant cultivars. Based on our results, the sugar beet cultivars such as Flores which was introduced in Iran as a cultivar

resistant to *R. solani* had considerable tolerance to charcoal rot as well (Tables 1 and 2). Therefore, these cultivars could be introduced to the farmers who face problem with the both diseases.

The fungus *M. phaseolina* is an important soil-borne pathogen with a wide range of hosts (Tomkins 1938) which has been isolated from such crops as corn, soybean, sesame, melon, beans, safflower and sugar beet in Iran (Ershad 2009). These crops are usually grown in rotation with sugar beet in different regions. The optimum temperature for crop infestation is often the same as that for crop growth. The aggressiveness of *Macrophomina* is intensified with increased temperature (Pearson et al. 1987) and water deficit stress. Thus, the disease occurs in almost all parts of the arid regions of Iran (Mahmoudi and Soltani 2005). The growth and development of the fungus is naturally quite fast in plant tissues depending to the internal status of the crop, so that it is slow in perennial and woody crops (Holliday and Punithalingam 1970).

Charcoal rot is known as a stress-related disease which injuries older plants under adverse environmental conditions. The heaviest infection occurs in months with a relatively higher temperature and moisture stress. The increase in the intensity of charcoal rot depends on the host, too (Beas-Fernandez et al. 2006). The study of favorable conditions for the incidence of charcoal rot in different crops like sugar beet indicates that hot and dry conditions cause the epidemics of this pathogen (Mayek-Perez 2002).One strategy to overcome this disease is to use resistant cultivars. Resistant or tolerant cultivars of such hosts as soybean have been identified in Iran (Raeyatpanah et al. 2007). In the case of sugar beet, the results showed that lines B8618, B8662 and B8751 could be used as pollinator parents for development of *M. phaseolina* and *R. solani* resistant cultivars.

Given the results of the evaluation of the resistance to *Rhizoctonia* and *Macrophomiana*, the line B8618 was found to be resistant to the both pathogens which can be explained by the similar disease-developing mechanisms of the two pathogens. These two pathogens used to be classified in one genus (Ashby 1927). This classification is due to similarity of the both pathogens with respect to their morphology, asexual reproduction, host range and disease symptom. Pectin lyase inhibitor protein existing in cell wall of sugar beet cultivars resistant to *R. solani* is known as a mechanism for resistance to the pathogen (Bugbee 1993). *M. phaseolina* also produces such enzymes during its entrance to the plant (Ahmad et al. 2006). Therefore, it is likely that there is a similar mechanism for resistance to *M. phaseolina* and *R. solani* in such resistant lines as B8618. Both pathogens produce appresorium and they penetrate into epidermal cells of the host by producing digestive enzymes (Amadioha 1998; Marcus et al. 1986).

The studied S1 lines had been obtained from two open-pollinated populations ("B" series. i.e. genotypes whose names start with B, from Bulk Shiraz and "M" series. i.e. genotypes whose names start with M, from BP Mashhad) which are resistant to *Rhizoctonia* and drought, respectively. Our results showed that although charcoal rot was prevailing in hot and arid conditions, drought-resistant lines (M293, M345 and M362) (Ahmadi et al. 2011) were not tolerant to this disease.

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